IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
Chatterjee, D.K.
Appl. No. 09/558,421
Filed: April 26, 2000
For: **Mutant DNA Polymerases and Uses Thereof**

Art Unit: 1652
Examiner: Rao, M.
Atty. Docket: 0942.3600003/RWE/BJD

Declaration of Kalavathy Sitaraman

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Kalavathy Sitaraman, do hereby declare and say:

1. THAT, I, Kalavathy Sitaraman, hold the degree of M.Sc. A recent copy of my Curriculum Vitae, accurately listing my scientific credentials and work experience, is attached hereto as Exhibit A.

2. THAT, since 1989, I have been employed by Life Technologies, Inc. (LTI) (and now Invitrogen Corporation)[1], the assignee of the above-captioned application, in the capacity of Associate Scientist III, Scientist, and Staff Scientist. *See* Exhibit A.

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[1]Life Technologies, Inc. merged with Invitrogen Corporation on September 12, 2000, with Invitrogen Corporation being the surviving entity.

3. THAT, during my employment by LTI (and now Invitrogen Corporation), I worked under the supervision of Dr. Deb K. Chatterjee on a project involving the cloning, expression, and characterization of wild-type and mutant DNA polymerases.

4. THAT, I have reviewed my laboratory notebooks detailing my work on the project. Based on these laboratory notebook records and my recollection, the following activities involving my work, and relating to the DNA polymerase project, took place during the period from about October 16, 1994, until about September 8, 1995.

On or about November 16, 1994, I performed an experiment testing the ability of certain primers to amplify GAPDH (glyceraldehyde-3-phosphate dehydrogenase). In this experiment, DeepVent® polymerase (plus and minus exonuclease activity), and various primers, were added to a DNA template. This activity was recorded on pages 96-97 of notebook 3831. A copy thereof is attached as Exhibit 1.

On or about November 18, 1994, I performed an experiment to determine the optimal concentrations of various polymerases to be used in amplifying DNA templates. In this experiment, various concentrations of *Taq*, DeepVent® and *Taq* + DeepVent® polymerases were added to a pMC9 DNA template. This activity was recorded on pages 99-100 of notebook 3831. A copy thereof is attached as Exhibit 2.

On or about November 21, 1994, I continued experiments to amplify pMC9. In this experiment, deoxyuracil-containing primers and a combination of *Taq* and DeepVent® polymerases were added to a pMC9 DNA template. She also tested Klenow *Taq* (KT) and

DeepVent® (DV) buffers by looking at the amount of product yielded by the reaction, as well as the fidelity of the primers. This experiment suggested that KT is a better buffer than DV and that a 1:0.01 unit ratio of *Taq* to DeepVent® is better than *Taq* alone. This activity was recorded on pages 101-102 of notebook 3831. A copy thereof is attached as Exhibit 3.

On or about November 21, 1994, I continued experiments to amplify pMC9. In this experiment, 1 unit of *Taq* and varying concentrations of DeepVent® polymerases were added to a pMC9 DNA template to determine optimal conditions for reducing or preventing mispriming. This activity was recorded on pages 103-104 of notebook 3831. A copy thereof is attached as Exhibit 4.

On or about November 21, 1994, I continued experiments to amplify pMC9. In this experiment, deoxyuracil-containing primers and varying concentrations of DeepVent® polymerase were added to a pMC9 DNA template. These results indicated that DeepVent® alone did not amplify the template under the tested conditions. This activity was recorded on page 105 of notebook 3831. A copy thereof is attached as Exhibit 5.

On or about November 22, 1994, I continued experiments to amplify pMC9. In this experiment, primers lacking deoxyuracil (dU) and *Taq*, DeepVent®, or *Taq* + DeepVent® polymerases were added to a pMC9 DNA template. These results confirmed that DeepVent® alone did not amplify template; the mispriming was virtually eliminated using *Taq* + DeepVent® polymerase with primers lacking dU; and that use of dU-containing primers resulted in the production of higher amounts of dimerized primers than did use of non-dU-containing primers.

This activity was recorded on page 106-108 of notebook 3831. A copy thereof is attached as Exhibit 6.

On or about November 28, 1994, I experimented with various polymerase chain reaction (PCR) conditions to prevent mispriming during pMC9 amplification. In this experiment, three different sets of primers were tested, and annealing temperatures and polymerase (*Taq*, DeepVent®, or *Taq* + DeepVent®) amounts were varied. This activity was recorded on page 109-110 of notebook 3831. A copy thereof is attached as Exhibit 7.

On or about November 29, 1994, I prepared and tested a new type of agarose for preparing gels. Samples tested were reaction products from the experiments described at pages 109-110 of notebook 3831 (*see* Exhibit 7). This activity was recorded on page 111 of notebook 3831. A copy thereof is attached as Exhibit 8.

On or about November 30, 1994, I continued experiments in which PCR conditions were varied, to determine optimal conditions for prevention of mispriming during pMC9 amplification. In this experiment, template concentrations, Mg^{++} concentrations, dNTP concentrations, and number of amplification cycles were varied during pMC9 amplification using *Taq*, DeepVent® or *Taq* + DeepVent® polymerases. This activity was recorded on pages 112-113 of notebook 3831. A copy thereof is attached as Exhibit 9.

On or about November 30, 1994, I conducted experiments in which pGAPDH was amplified using *Taq*, DeepVent® and *Taq* + DeepVent® polymerases. The results suggested that

DeepVent® alone at low concentrations gave the best product yield. This activity was recorded on pages 114-115 of notebook 3831. A copy thereof is attached as Exhibit 10.

On or about December 1, 1994, I continued experiments in which pMC9 was amplified. In this experiment, new deoxyuracil-containing primers were tested and Mg^{++} concentrations were varied to optimize reaction conditions that would prevent the mispriming of the pMC9 template. This activity was recorded on pages 116-117 of notebook 3831. A copy thereof is attached as Exhibit 11.

On or about December 1, 1994, I continued experiments to optimize the conditions for amplifying pMC9. In this experiment, the concentration of *Taq* polymerase was increased from 1 unit to 2 units. This activity was recorded on page 118 of notebook 3831. A copy thereof is attached as Exhibit 12.

On or about December 4, 1994, I performed experiments to determine optimal reaction conditions for UDG cloning of PCR reaction products. Concentrations of UDG and PCR products were titrated and the reaction mixtures were used to transform MaxEfficiency® DH5α *E. coli* host cells. This activity was recorded on pages 120-121 of notebook 3831. A copy thereof is attached as Exhibit 13.

On or about December 6, 1994, I continued experiments to optimize the conditions for amplification of pMC9. In this experiment, 2 units of *Taq* or *Taq* + DeepVent® polymerases and non-deoxyuracil forward and reverse primers, were used to amplify pMC9. This activity was recorded on pages 122-123 of notebook 3831. A copy thereof is attached as Exhibit 14.

On or about December 6, 1994, I prepared the pUC19 vector. In this experiment, the vector was digested with the AatII, EcoRI, BamHI, and AflIII restriction enzymes. A diagnostic gel was run to ensure the proper restriction pattern. Unrestricted pUC19 was gel-purified and ethanol-precipitated. This activity was recorded on pages 124-125 of notebook 3831. A copy thereof is attached as Exhibit 15.

On or about December 7, 1994, I performed experiments to transform bacterial cells with pUC19 vector amplified using forward and reverse non-deoxyuracil primers, and varying amounts of either *Taq* or *Taq* + DeepVent® polymerases. This activity was recorded on page 126 of notebook 3831. A copy thereof is attached as Exhibit 16.

On or about December 8, 1994, I performed experiments to amplify pUC19 vector that had been linearize by digestion with XmaI. Amplification was performed with either *Taq* or *Taq* + DeepVent® polymerases, and the concentration of Mg^{++} was titrated in the reaction mixtures. The PCR products from duplicate samples were pooled, phenol-extracted and ethanol precipitated. The amount of DNA was then quantified on a gel, · DNA was then cut with a restriction enzyme, and an 875 bp insert was gel-purified. This activity was recorded on pages 127-130 of notebook 3831. A copy thereof is attached as Exhibit 17.

On or about December 12, 1994, I performed experiments to ligate the purified vector and insert from the experiment conducted on December 8, 1994 (*see* Exhibit 17). Vector and insert were amplified in the presence of *Taq* or *Taq* + DeepVent® polymerases, or in the absence of polymerase (controls). *E. coli* DH5α MaxEfficiency® cells were transformed with the ligated

products. This activity was recorded on page 131 of notebook 3831. A copy thereof is attached as Exhibit 18.

On or about December 13, 1994, I ran the vector, insert and ligation reaction products (*see* Exhibit 18) on a gel to determine if the ligation was successful. Bacteria were re-transformed with the products of the *Taq* and *Taq* + DeepVent® reactions and replated. Mini-preps were prepared from colonies picked from plates that had received *Taq* or *Taq* + DeepVent® samples. This activity was recorded on pages 132-133 of notebook 3831. A copy thereof is attached as Exhibit 19.

On or about December 13, 1994, I continued experiments to optimize conditions for ligation of vector and insert. The ligation reaction was performed with a different ratio of vector to insert from that in the previous experiments, and *E. coli* DH5α cells were transformed with the product. This activity was recorded on page 134 of notebook 3831. A copy thereof is attached as Exhibit 20.

On or about December 14, 1994, I replated the transformed cells left over from the December 13, 1994, transformation reaction (*see* Exhibit 20). This activity was recorded on page 135 of notebook 3831. A copy thereof is attached as Exhibit 21.

On or about December 14, 1994, I used inbalance reaction mixtures from Ayoub Rashtchian, another employee of LTI, in the transformation step, to determine if better transformant/mutant colony numbers could be obtained. This activity was recorded on pages 136-137 of notebook 3831. A copy thereof is attached as Exhibit 22.

On or about December 16, 1994, I counted blue and white colonies to determine transformation efficiency from the *Taq* and *Taq* + DeepVent® reactions. This activity was recorded on page 138 of notebook 3831. A copy thereof is attached as Exhibit 23.

On or about December 16, 1994, I performed experiments to determine if all of the white colonies obtained in the previous experiment (*see* Exhibit 23) were full-length clones, by miniprepping overnight cultures prepared from white colonies picked from the previous experiment. This activity was recorded on page 139-140 of notebook 3831. A copy thereof is attached as Exhibit 24.

On or about December 19, 1994, I continued experiments to amplify pMC9, to determine fidelity of amplification with varying amounts of *Taq* or *Taq* + DeepVent® polymerases. In this experiment, the concentration of template was varied, and template was amplified using 1 or 2 units of polymerase. This activity was recorded on pages 141-142 of notebook 3831. A copy thereof is attached as Exhibit 25.

On or about December 20, 1994, I continued experiments to optimize the conditions for amplifying pMC9, using two units of *Taq* polymerase and varying concentrations of DeepVent® polymerase. This activity was recorded on page 143 of notebook 3831. A copy thereof is attached as Exhibit 26.

On or about December 27, 1994, I reran the samples from the December 20-22, 1994, experiment (*see* Exhibit 26) on an agarose gel. This activity was recorded on page 144 of notebook 3831. A copy thereof is attached as Exhibit 27.

On or about December 27, 1994, I repeated the experiment from December 20-22, 1994 (*see* Exhibit 26), by attempting to amplify pMC9 using 2 units of *Taq* polymerase and varying concentrations of DeepVent® polymerase. This activity was recorded on pages 145-146 of notebook 3831. A copy thereof is attached as Exhibit 28.

On or about December 27, 1994, I continued experiments to amplify pMC9 using varying ratios of *Taq* + DeepVent® polymerases or varying concentrations of *Taq* or DeepVent® polymerase alone. This activity was recorded on page 147 of notebook 3831. A copy thereof is attached as Exhibit 29.

On or about December 27, 1994, I continued experiments to amplify pMC9 using *Taq* + DeepVent® polymerases in a ratio of 2 units of *Taq* polymerase to 0.2 units of DeepVent® polymerase and varying concentrations of Mg^{++}. This activity was recorded on pages 148 of notebook 3831. A copy thereof is attached as Exhibit 30.

On or about December 28, 1994, to January 3, 1995, I continued experiments to amplify pMC9 using varying concentrations of *Taq* and DeepVent® polymerases. The effect of freshly preparing the enzyme mixture and adding it separately was also determined. This activity was recorded on pages 149-150 of notebook 3831. A copy thereof is attached as Exhibit 31.

On or about January 3-4, 1995, I pooled the products from the December 27 and December 28 reactions (*see* Exhibits 28 through 31), and extracted and purified the DNA. This activity was recorded on page 151 of notebook 3831. A copy thereof is attached as Exhibit 32.

On or about January 3-5, 1995, I started bacterial cultures to propagate different sized fragments of human genomic sequence in pDELTA1. This activity was recorded on page 152 of notebook 3831. A copy thereof is attached as Exhibit 33.

On or about January 5, 1995, I attempted to amplify the 6.4 kb and 8.0 kb fragments using *Taq* and DeepVent® polymerases at a 1:0.01 concentration ratio. This activity was recorded on pages 153-154 of notebook 3831. A copy thereof is attached as Exhibit 34.

On or about January 5, 1995, I attempted to optimize conditions for the amplification of the 6.4 kb fragment using *Taq* polymerase alone, or mixtures of *Taq* + DeepVent® polymerases at varying concentration ratios. This activity was recorded on pages 155-158 of notebook 3831. A copy thereof is attached as Exhibit 35.

On or about January 9, 1995, I attempted to amplify the 10.5 kb fragment contained in pDELTA1, using varying amounts of *Taq* + DeepVent® polymerases and varying concentrations of Mg^{++}. This activity was recorded on pages 159-160 of notebook 3831. A copy thereof is attached as Exhibit 36.

On or about January 9, 1995, I grew bacterial host cells that had been transformed with amplified fragments of different sizes, under more stringent conditions, in order to decrease the number of bacterial colonies on each plate. This activity was recorded on page 161 of notebook 3831. A copy thereof is attached as Exhibit 37.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any registration resulting therefrom.

Further, declarant sayeth not.

Date: 12/3/01

Name: Kalavathy Sitaraman

Signature: Kalavathy Sitaraman

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SKGF1/25/98 dcw

Exhibit A
Appl. No. 09/558,421

KALAVATHY SITARAMAN

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Summary:

Staff Scientist with 13 years of R&D and Manufacturing experience in biotechnology industry and seven years of academic research. Strong scientific and technical skills in the field of molecular biology. Extensive experience in assay development as an applications scientist. Strengths include excellent multitasking and troubleshooting skills. Capable of independent research and a great team player. Confident, self motivated, proactive with proven track record of exceeding personal and corporate objectives.

Professional Experience:

LIFE TECHNOLOGIES, A DIVISION OF INVITROGEN CORPORATION **1989 - Present**

Staff Scientist/Manufacturing Technologies, Invitrogen, Frederick, MD

- Process improvement, assay development and troubleshooting of multi-million dollar amplification products line.
- Technical liaison between R&D and Operations. New product transfer to manufacturing.
- Validated scale-up processes to insure stability, accuracy, consistency and reproducibility of PCR products.
- Scientific and technical direction to production and quality control scientists for efficient manufacturing and timely release of amplification products.
- Manufacturing representative for all amplification products team.

Scientist/Manufacturing Site Technical Support, Invitrogen, Frederick, MD

- Day-to-day troubleshooting of manufacturing and Quality Control of amplification products.
- Trained and mentored junior scientists on PCR QC applications.
- Developed and implemented manufacturing documents including scientific SOPs, production and QC protocols.
- Developed protocols and performed long-term stability assays for DNA/RNA polymerases and antibodies.
- New product transfers from R&D to operations.

Scientist/R&D, Molecular Biology, Life Technologies, Rockville, MD

- Research and Development of PCR, RT-PCR, Sequencing and UltraPure products.
- Applications Scientist: Developed protocols and published data for PCR applications.
- Prepared development reports, QC protocols and contributed to several new product proposals.
- Evaluated different methods, conducted feasibility studies, performed competitive audit for amplification, sequencing and UltraPure products.
- Represented R&D amplification and sequencing team and participated in marketing product shows.

Research Assistant II, University of Connecticut, Storrs, CT **1986 - 1988**

Department of Molecular and Cell Biology and Department of Plant Physiology

- Research: Drosophila Genetics - Transposable P elements and male recombination.
- Research: Plant Protoplast isolation.

Laboratory Technician, Purdue University, West Lafayette, IN **1982 - 1986**

Department of Botany & Plant Pathology and Department of Biological Sciences:

- Research: Corn Genetics and Biochemistry - Translational alterations in maize responding to different stresses.
- Research: Plant Virology - Protein-RNA interactions.

Education:

M.Sc, Zoology (specialization in Entomology and Nematology), Madurai University, India.
B.Sc, Zoology (minor in Botany and Chemistry), Madras University, India.

Workshops and Training:

- Life Technologies workshops on:
  Gateway Cloning, Recombinant Bacculovirus Expression
  Recombinant DNA, PCR and Advanced PCR, In-situ Hybridization Techniques
  cDNA library, Cell Culture Techniques
- Hands-on cross training in large scale DNA and Protein Purification (Life Technologies)
- J.D. Edwards General Overview (Life Technologies)
- cGMP and New Quality System Regulations Certification (Life Technologies)
- Differential Display (Gene Hunter, Nashville)
- Capillary Electrophoresis (Beckman)
- Science of Freeze Drying (PAFRA Biopreservations)
- TechLine Certification (Life Technologies)

Research and Technical Skills:

- Molecular Biology: 20 years of experience in academic and biotechnology industry.
  Nucleic acid: DNA /RNA isolation and purification, hybridization and detection.
  Cloning: Vector construction, bacterial transformation, and mutagenesis.
  Amplification: PCR, RT-PCR and Geno-typing.
  Sequencing: Manual and automated.
- Protein Chemistry: Kinetic and enzymatic functional assays, electrophoresis, filtration and spectrophotometry.
- Tissue Culture: Sterile Techniques, cell culture, selection and growth of many types of bacteria.
- Fermentation: Large scale bacterial fermentation.
- Laboratory Management:
  Supervisory: Mentoring of junior scientists and Training peers in molecular biology applications.
  Assignment of work to junior scientists, making decisions on QC.
  Administrative: Supply and inventory management.
- Scientific and technical writing: Publication articles, SOP, Development reports, QC Protocols, Product Profiles.
- Computer: Working knowledge of Microsoft Word, Excel and PowerPoint
  Clone Manager, Oligo, Align, Primer, GeneScan, DNA Star.

Publications:

- 13 articles in Life Technologies, FOCUS (1991 - 2000) and 1 Application Note in American Biotechnology Laboratory (1997).
- Two publications in Journal of Genetics (1990).
- Four publications in leading journals including Virology, Biochemistry, Plant Physiology (1983 - 1987).

Honors and Awards:

- Employee Achievement Award, Invitrogen, Manufacturing Technologies, 2001.
- Employee Achievement Award, Life Technologies, Technical support, 2000.
- Award of Excellence, Life Technologies, R&D, 1997, 1998 and 1999.

Professional Affiliation:

Protein Society
Science Advisory Board